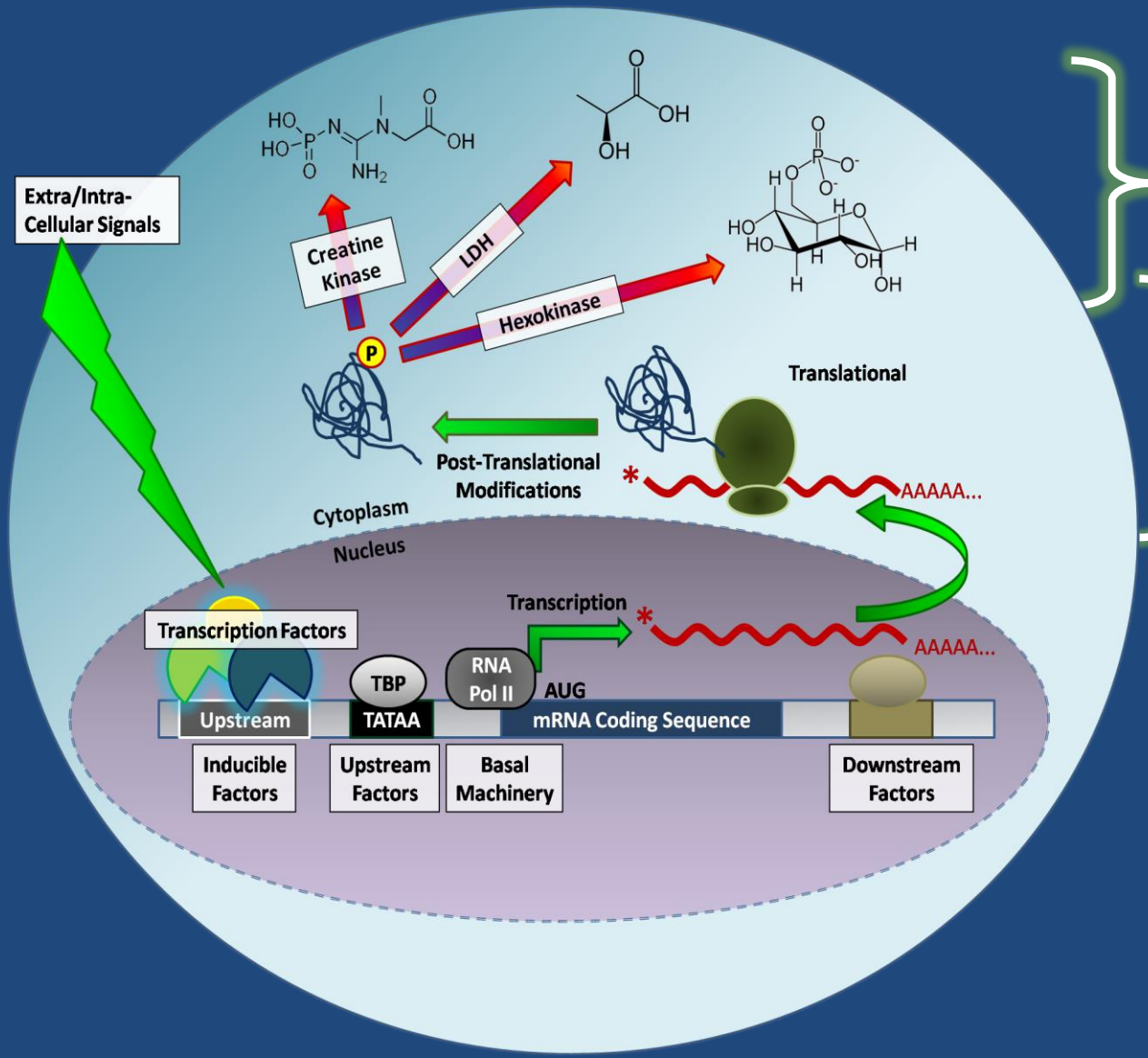
INSECT COLD HARDINESS : A MOLECULAR TOOLKIT

www.carleton.ca/~kbstorey

Introduction to “Omics”



Metabolomics

Proteomics

Genomics

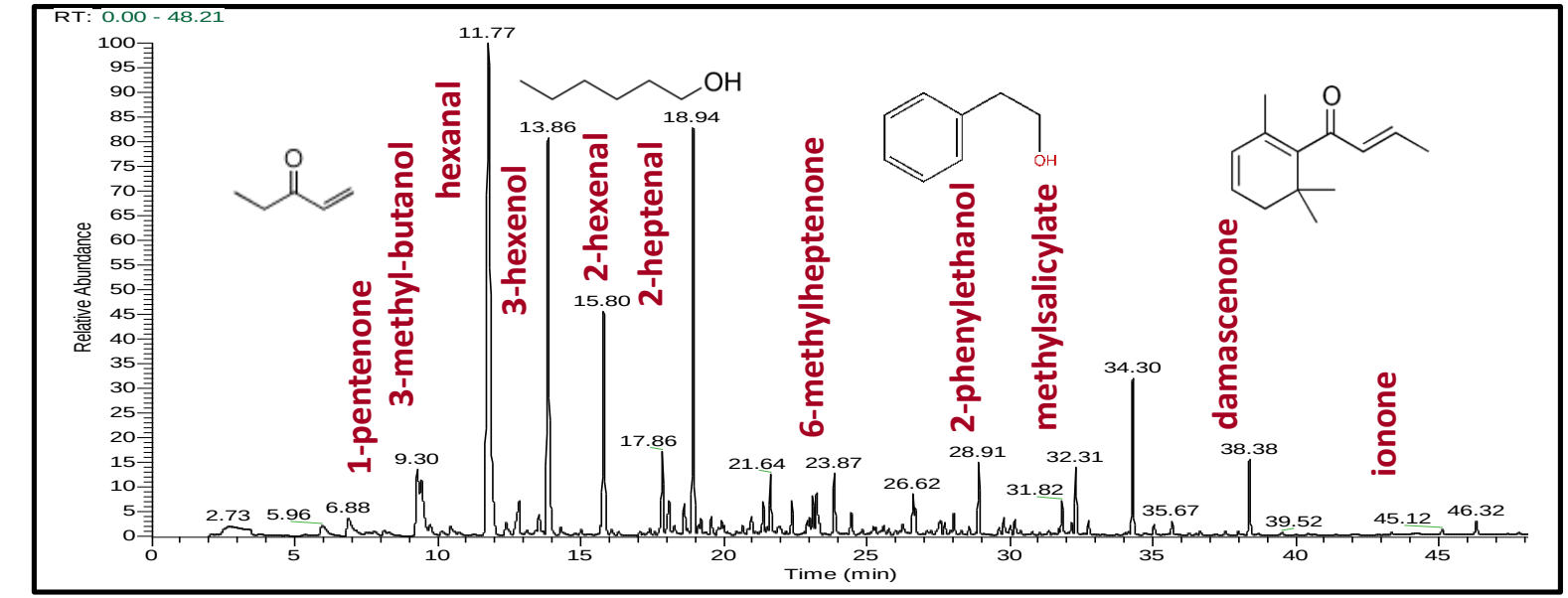
Metabolomics: Profiling

Separation methods

- Gas chromatography (GC)
- High performance liquid chromatography (HPLC)
- Capillary electrophoresis (CE)

Detection methods

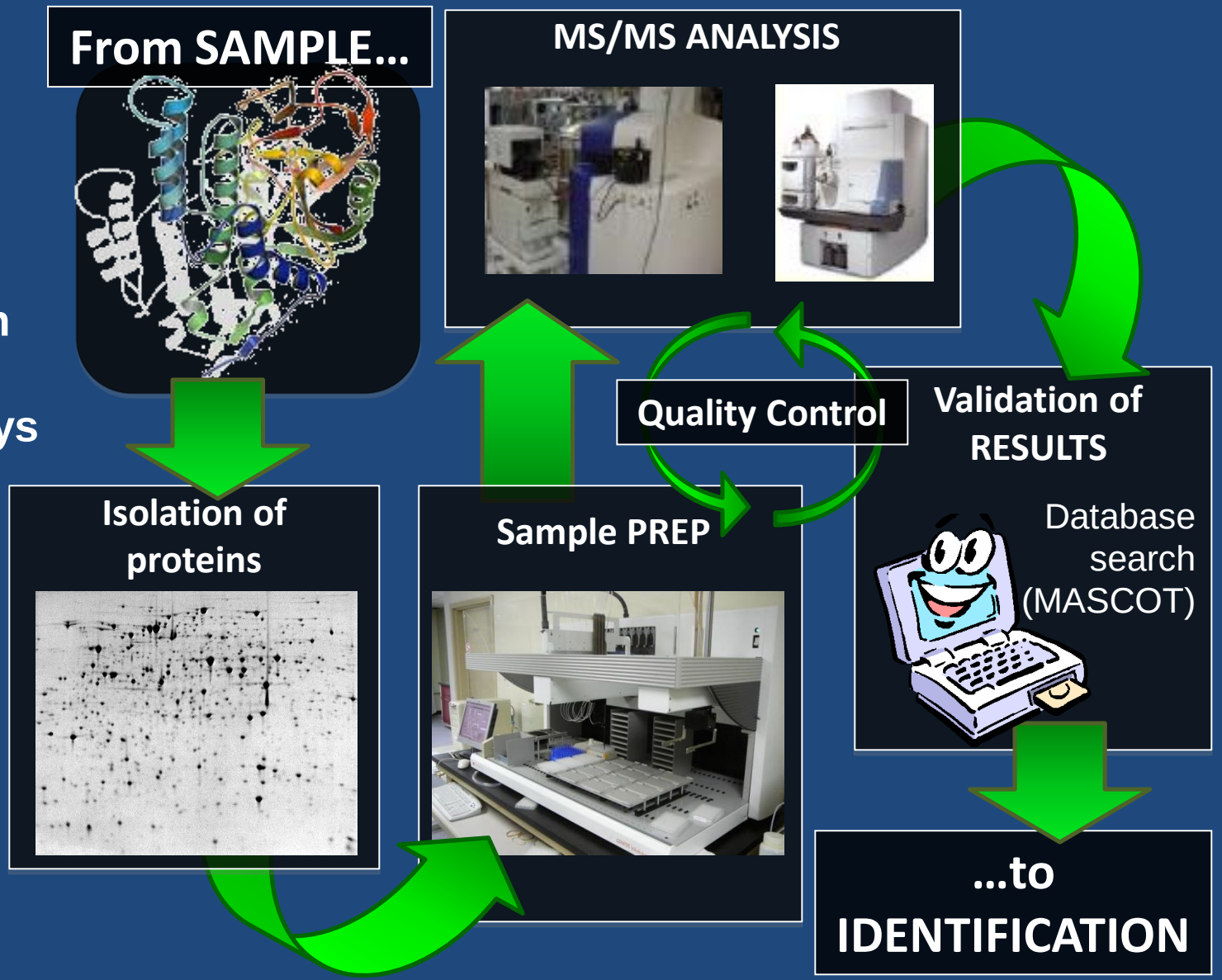
- Mass spectrometry (MS)
- Nuclear magnetic resonance (NMR) spectroscopy



Proteomics: Discovery

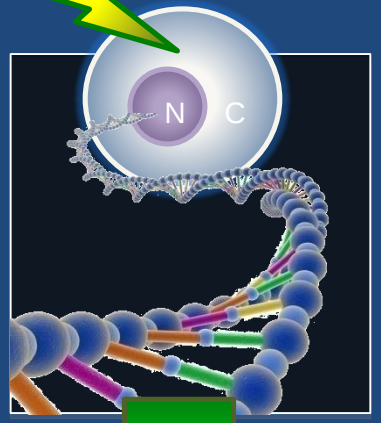
METHODS

- In vivo detection
- In situ localization
- Co-IP
- Protein Chip Arrays
- 2D PAGE



Genomics

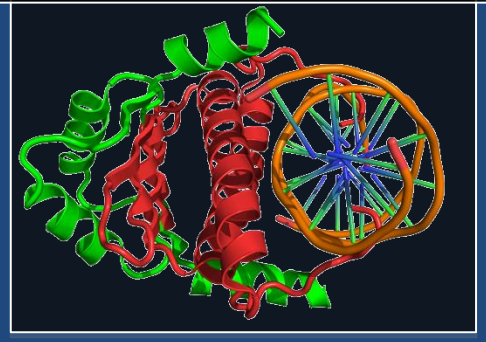
STRESS



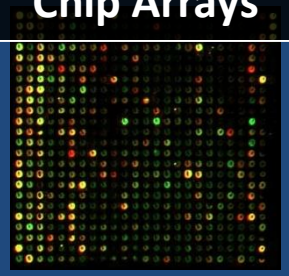
mRNA Transcript

AAA...

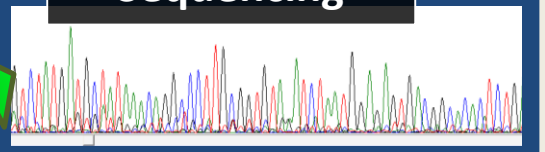
Transcription Factor



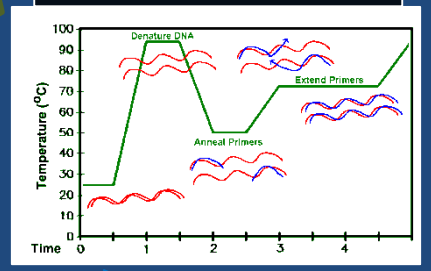
Chip Arrays



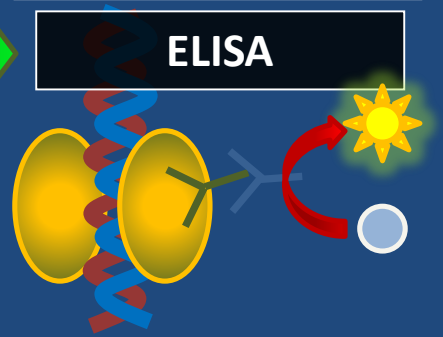
Sequencing



RT-PCR/RACE



ELISA



SURVIVAL

CHARACTERIZE GENE REGULATORY NETWORKS

Turning it all off

© 2015. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2015) 218, 1281-1289 doi:10.1242/jeb.104828

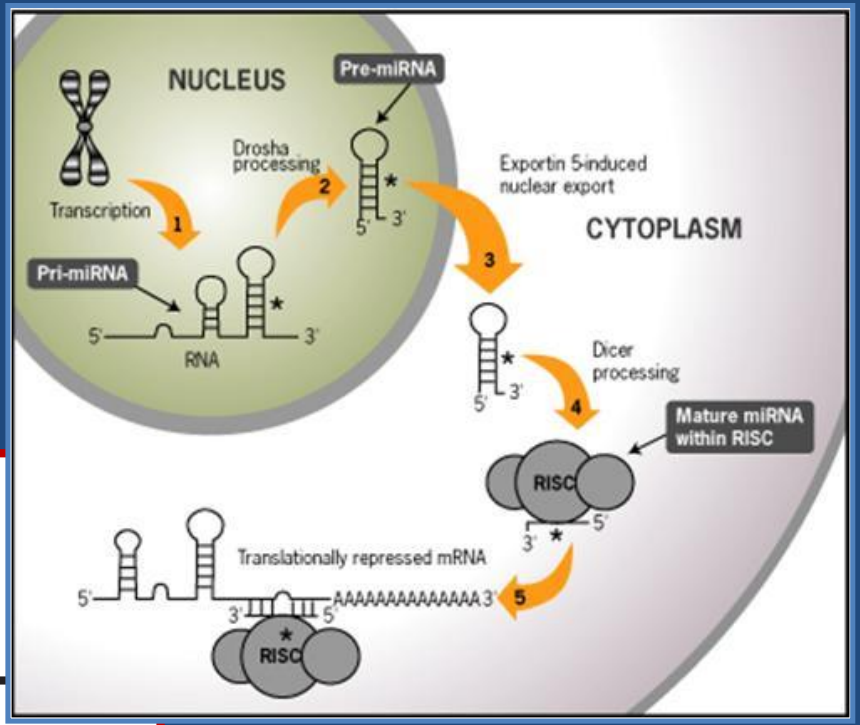
COMMENTARY

Insight into post-transcriptional gene regulation: stress-responsive microRNAs and their role in the environmental stress survival of tolerant animals

Kyle K. Biggar[1] and Kenneth B. Storey[2,*]

ABSTRACT

Living animals are constantly faced with various environmental stresses that challenge normal life, including: oxygen limitation, very low or high temperature, as well as restriction of water and food. It has been well established that in response to these stresses, tolerant organisms regularly respond with a distinct suite of cellular modifications that involve transcriptional, translational and post-translational modification. In recent years, a new mechanism of rapid and reversible transcriptome regulation, via the action of non-coding RNA molecules, has emerged into post-transcriptional regulation and has since been shown to be part of the survival response. However, these RNA-based mechanisms by which tolerant organisms respond to stressed conditions are not well understood. Recent studies have begun to show that non-coding RNAs control gene expression and translation of mRNA to protein, and can also have regulatory influence over major cellular processes. For example, select microRNAs have been shown to have regulatory influence over the cell cycle, apoptosis, signal transduction, muscle atrophy and fatty acid metabolism during periods of environmental stress. As we are on the verge of dissecting the roles of non-coding RNA in environmental stress adaptation, this Commentary summarizes the hallmark alterations in microRNA expression that facilitate stress survival.



Biochimica et Biophysica Acta 1779 (2008) 628–633

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Differential expression of microRNA species in organs of hibernating ground squirrels: A role in translational suppression during torpor

Pier Jr. Morin, Adrian Dubuc, Kenneth B. Storey *

Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

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MicroRNA
Hibernation
Spermophilus tridecemlineatus
Dicer
Reversible control of translation

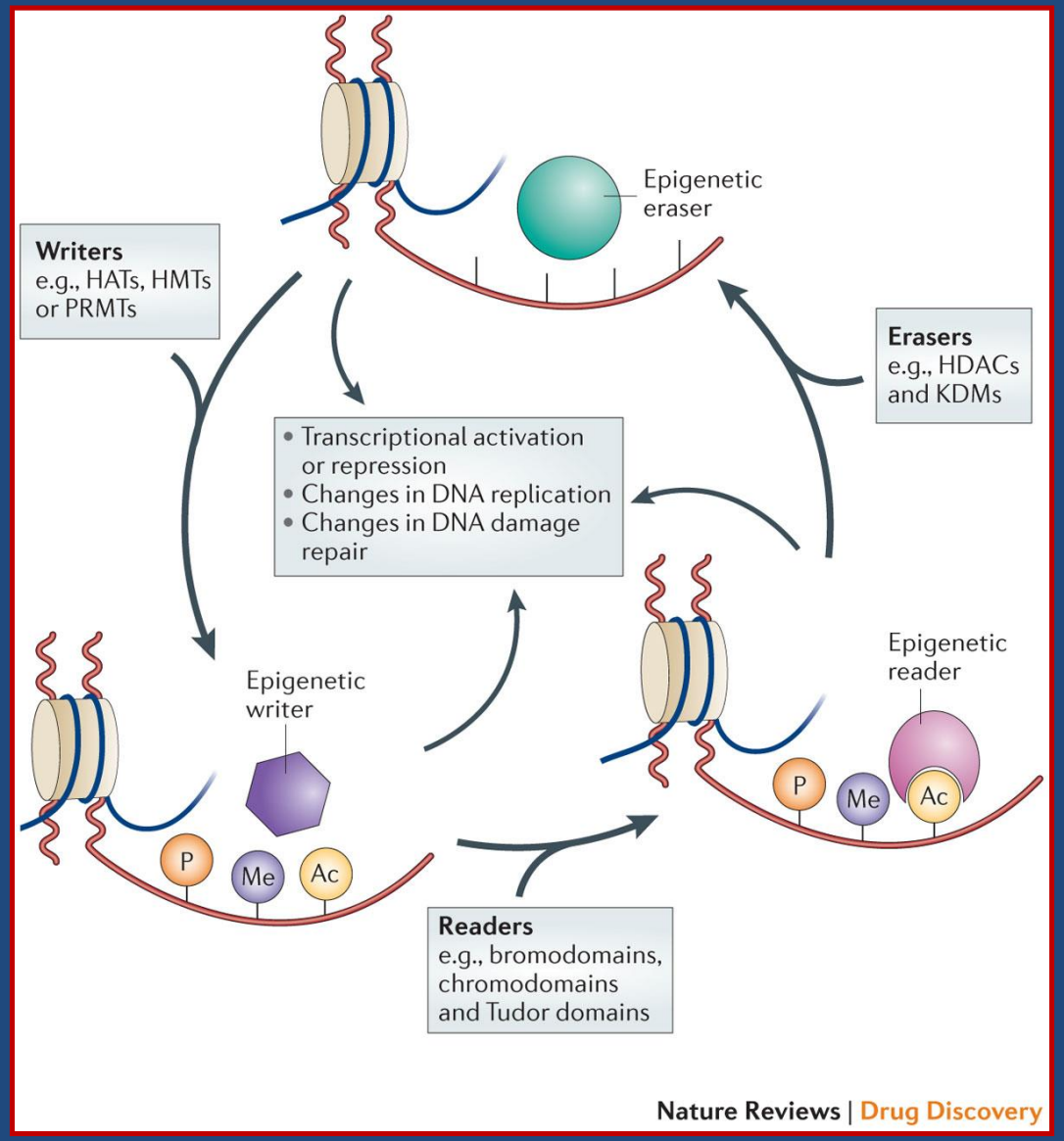
ABSTRACT

Mammalian hibernation includes long periods of profound torpor where the rates of all metabolic processes are strongly suppressed in a reversible manner. We hypothesized that microRNAs (miRNAs), small non-coding transcripts that bind to mRNA, could play a role in the global suppression of mRNA translation when animals enter torpor. Selected miRNA species (4–9 of the following: mir-1, mir-24, mir-15a, mir-16, mir-21, mir-122a, mir-143, mir-146 and mir-206) were evaluated in four organs of euthermic versus hibernating ground squirrels, *Spermophilus tridecemlineatus* using RT-PCR. Levels of mir-24 transcripts were significantly reduced in heart and skeletal muscle of torpid animals as were mir-122a levels in the muscle. Mir-1 and mir-21 both increased significantly in kidney during torpor by 2.0- and 1.3-fold, respectively. No changes were found for the four miRNA species analyzed in liver. Protein levels of Dicer, an enzyme involved in miRNA processing were also quantified in heart, kidney and liver. Dicer protein levels increased by 2.7-fold in heart

EPIGENETIC MECHANISMS

Master Switch:
CHANGE THE READING of YOUR DNA

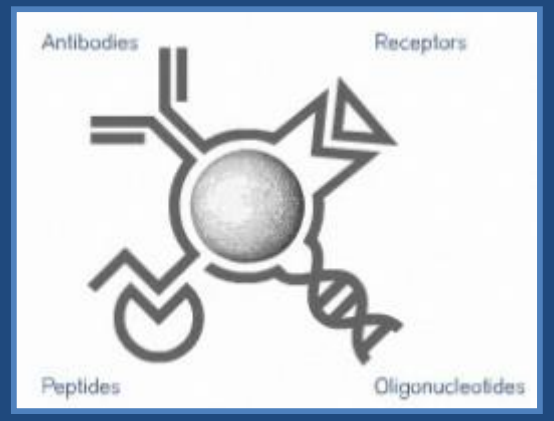
Turn Genes On and OFF:
in response to environment [Disease, Lifestyle, Drugs, Interventions]

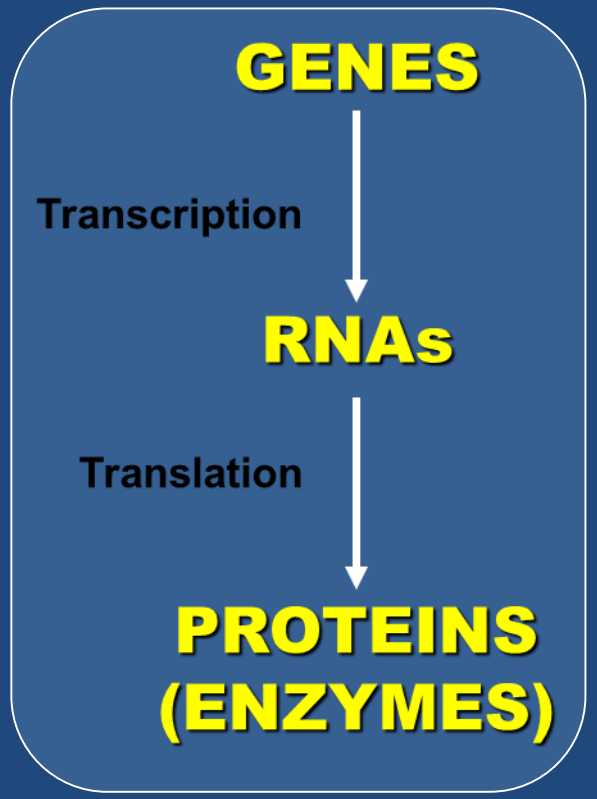


Multiplex technology

- Quantitatively measure multiple analytes in a single assay
  - -- i.e. 3-50 protein targets in 1 well
- Primarily nucleic acid and protein–based techniques
- Luminex: crucial type of MULTIPLEX technology

Applications

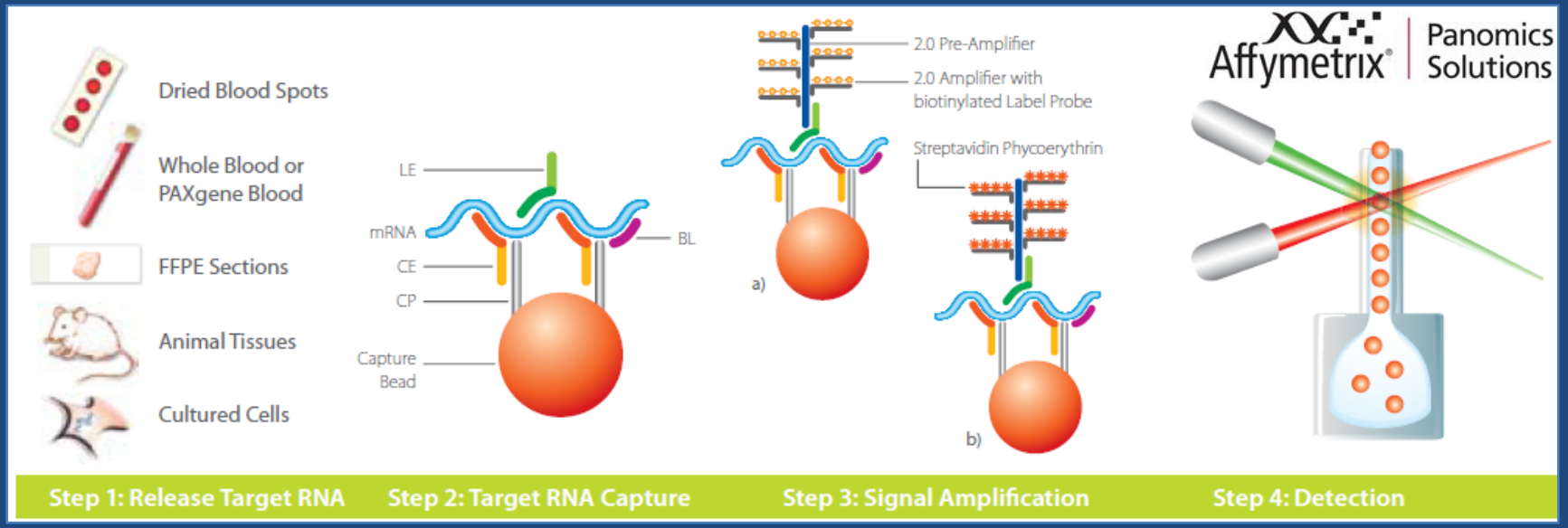




- Covalent attachment of: Antibodies, Oligonucleotides
- Capture of proteins, peptides, coding and non-coding RNAs, miRNA targets and more !
- Create a snapshot of Global Cellular Functions

Use this ‘snapshot’ to identify mechanisms of metabolic regulation

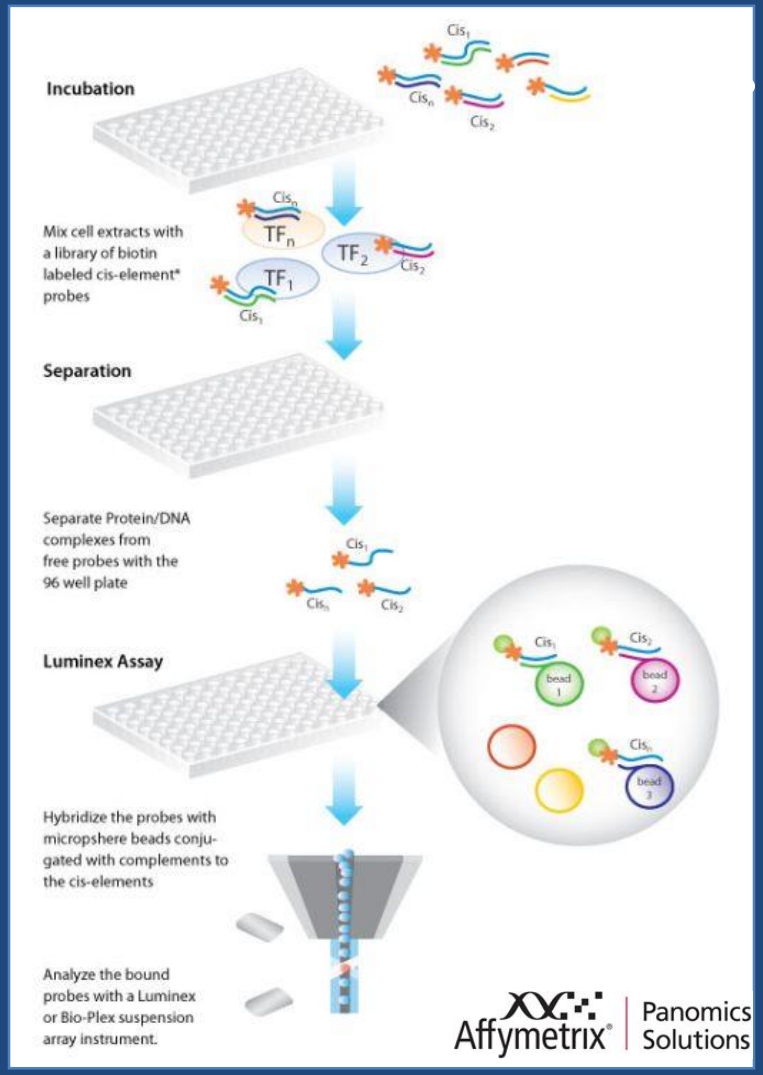
mRNA applications



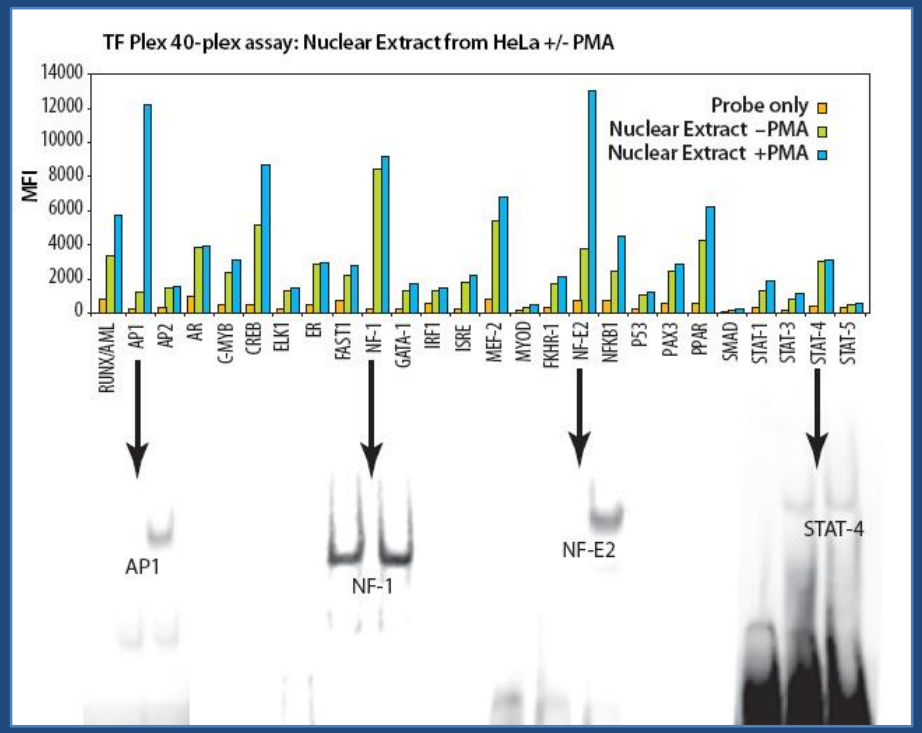
- QuantiGene Plex
- Same technology: Immobilized oligonucleotides
- Direct measure of mRNA levels
- Custom-plex can measure 3-80 genes in 1 sample

Transcription Factor ELISA

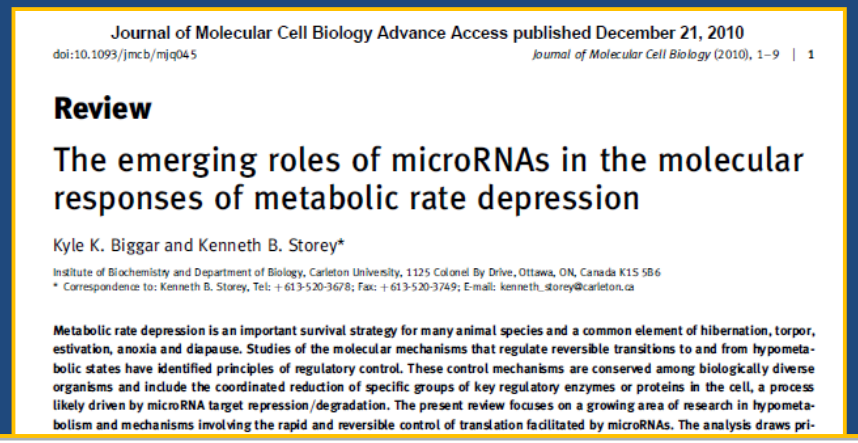
Procarta® TF Plex Assays: Luminex based



Profile the DNA binding activity of up to **44 different** transcription factors (TFs) in a single well



miRNA: Multiplex them all



Journal of Molecular Cell Biology Advance Access published December 21, 2010

doi:10.1093/jmcb/mjq045 *Journal of Molecular Cell Biology* (2010), 1–9 | 1

Review

The emerging roles of microRNAs in the molecular responses of metabolic rate depression

Kyle K. Biggar and Kenneth B. Storey*

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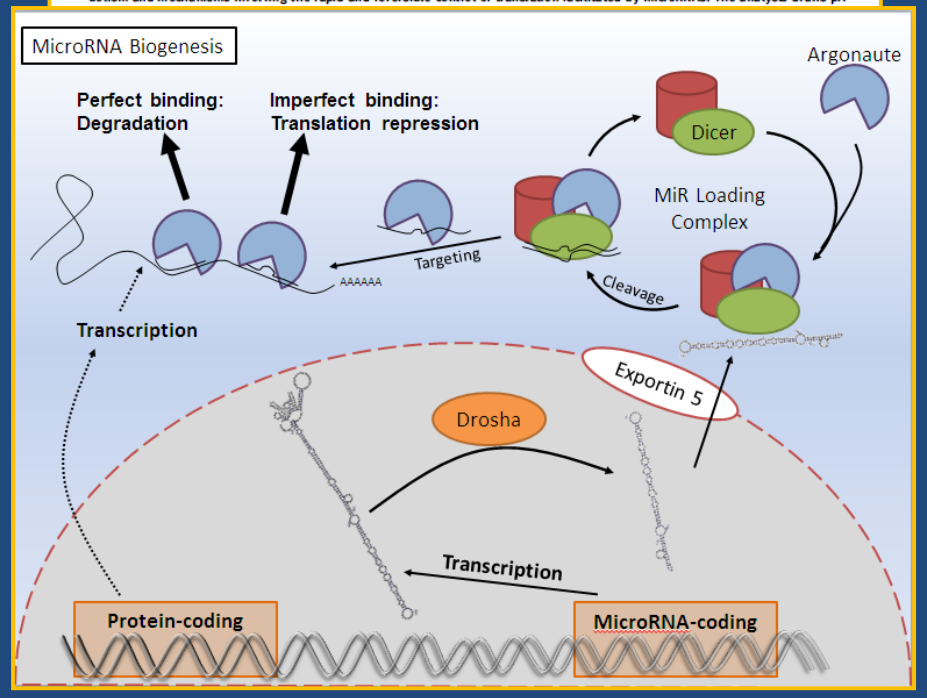
* Correspondence to: Kenneth B. Storey, Tel: +613-520-3678; Fax: +613-520-3749; E-mail: kenneth_storey@carleton.ca

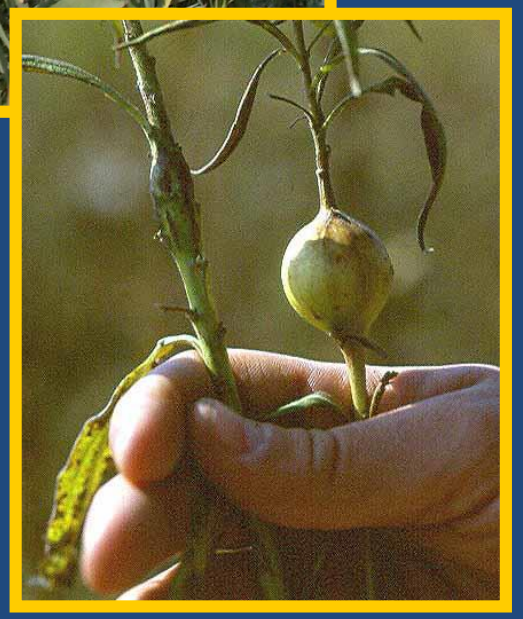
Metabolic rate depression is an important survival strategy for many animal species and a common element of hibernation, torpor, estivation, anoxia and diapause. Studies of the molecular mechanisms that regulate reversible transitions to and from hypometabolic states have identified principles of regulatory control. These control mechanisms are conserved among biologically diverse organisms and include the coordinated reduction of specific groups of key regulatory enzymes or proteins in the cell, a process likely driven by microRNA target repression/degradation. The present review focuses on a growing area of research in hypometabolism and mechanisms involving the rapid and reversible control of translation facilitated by microRNAs. The analysis draws pri-



- Size ~22 nucleotides
- Highly conserved across species
- Bind to 3' UTR of mRNAs
- Exact repression mechanism(s) yet to be defined, but seem to include

  - Block translation of mRNA
  - Help bind mRNA into stress granules
  - Target mRNA for degradation

GOLDENROD GALL INSECTS



SUMMER

WINTER

Eurosta solidaginis -
the goldenrod gall fly

Ball gall

FREEZE TOLERANT

Mature 3rd instar larva overwinters

Larva

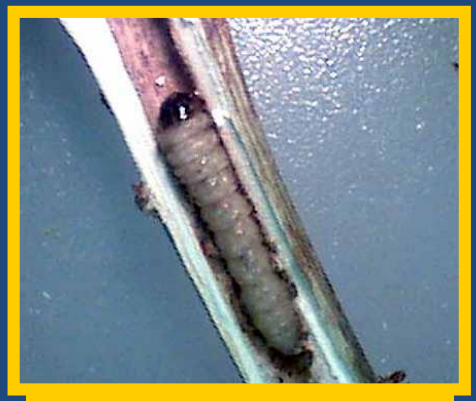
Gall interior

Adult fly

Epiblema scudderiana - the goldenrod gall moth

Elliptical gall

FREEZE AVOIDING



Mature larva overwinters

Larva

Gall interior

Adult moth

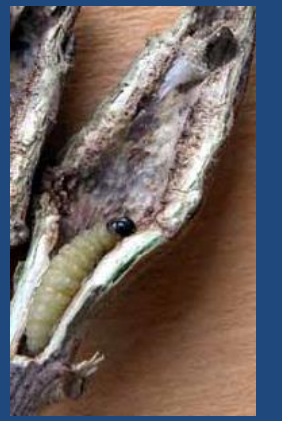
GOLDENROD GALL INSECTS

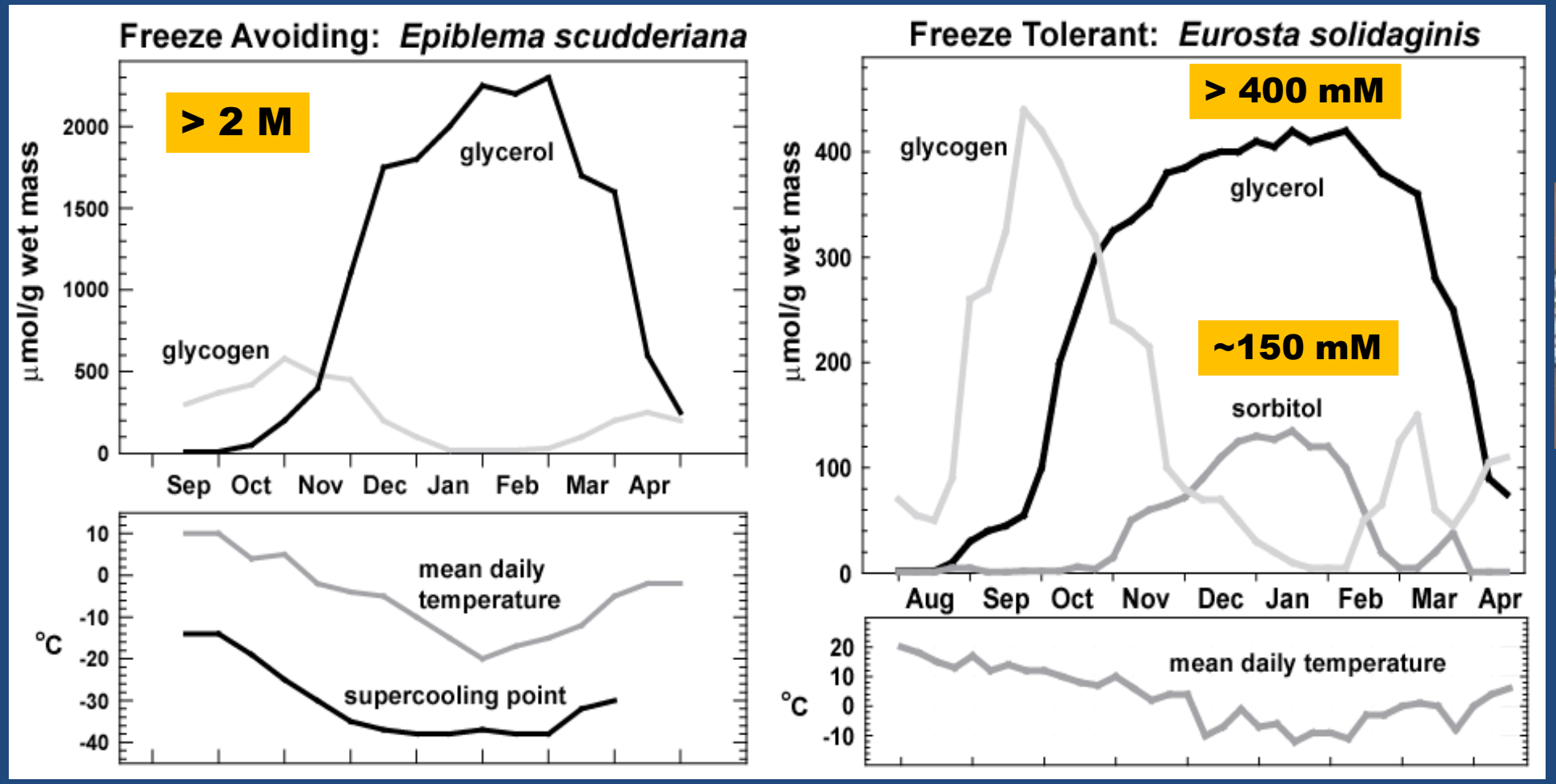
Epiblema scudderiana
(Clemens)
(Lepidoptera, Olethreutidae)

Eurosta solidaginis
(Fitch)
(Diptera, Tephritidae)

Freeze Avoiding		**Freeze Tolerant**
-38°C	SCP	-8°C
0	% Ice	67%
25%	Water loss	-
Antifreeze proteins	Ice control	Ice nucleators
Glycerol	Polyols	Glycerol, Sorbitol

Winter profiles of cold hardening in 2 insects





FREEZE AVOIDING

FREEZE TOLERANT

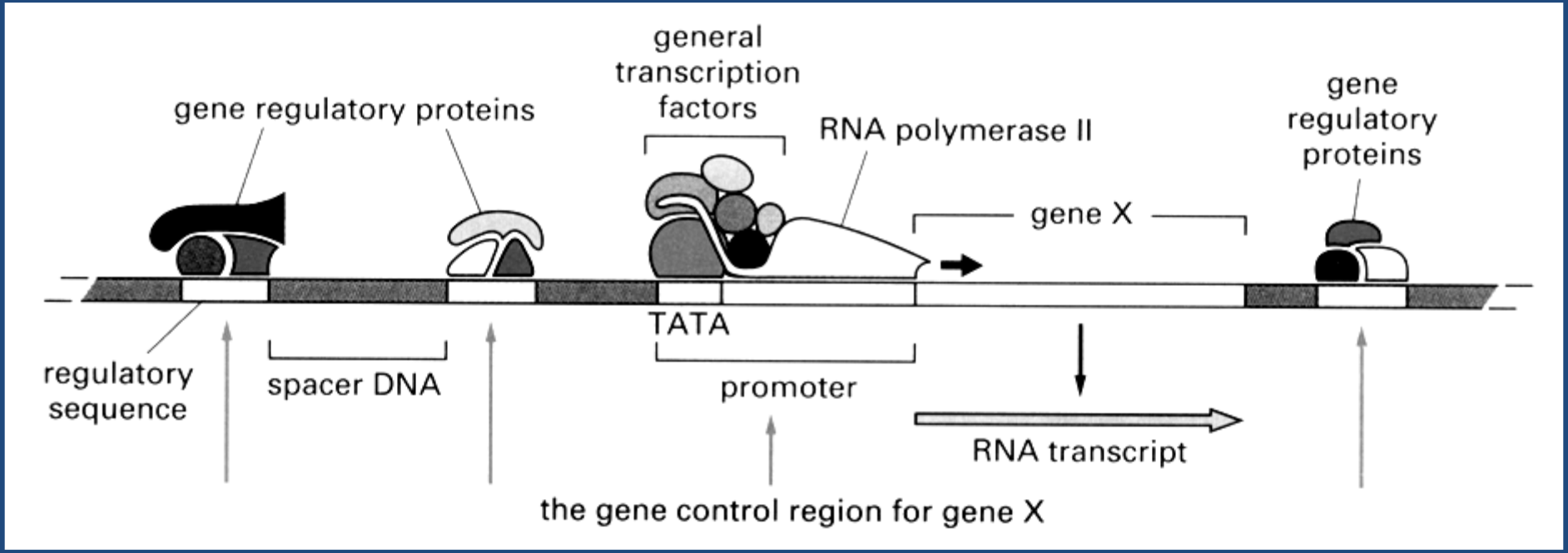
EUROSTA HIF-1α

***Drosophila* HIF-1α called Sima**
(HIF-1β = ARNT = Tango)

***Eurosta* has 62% identity overall**

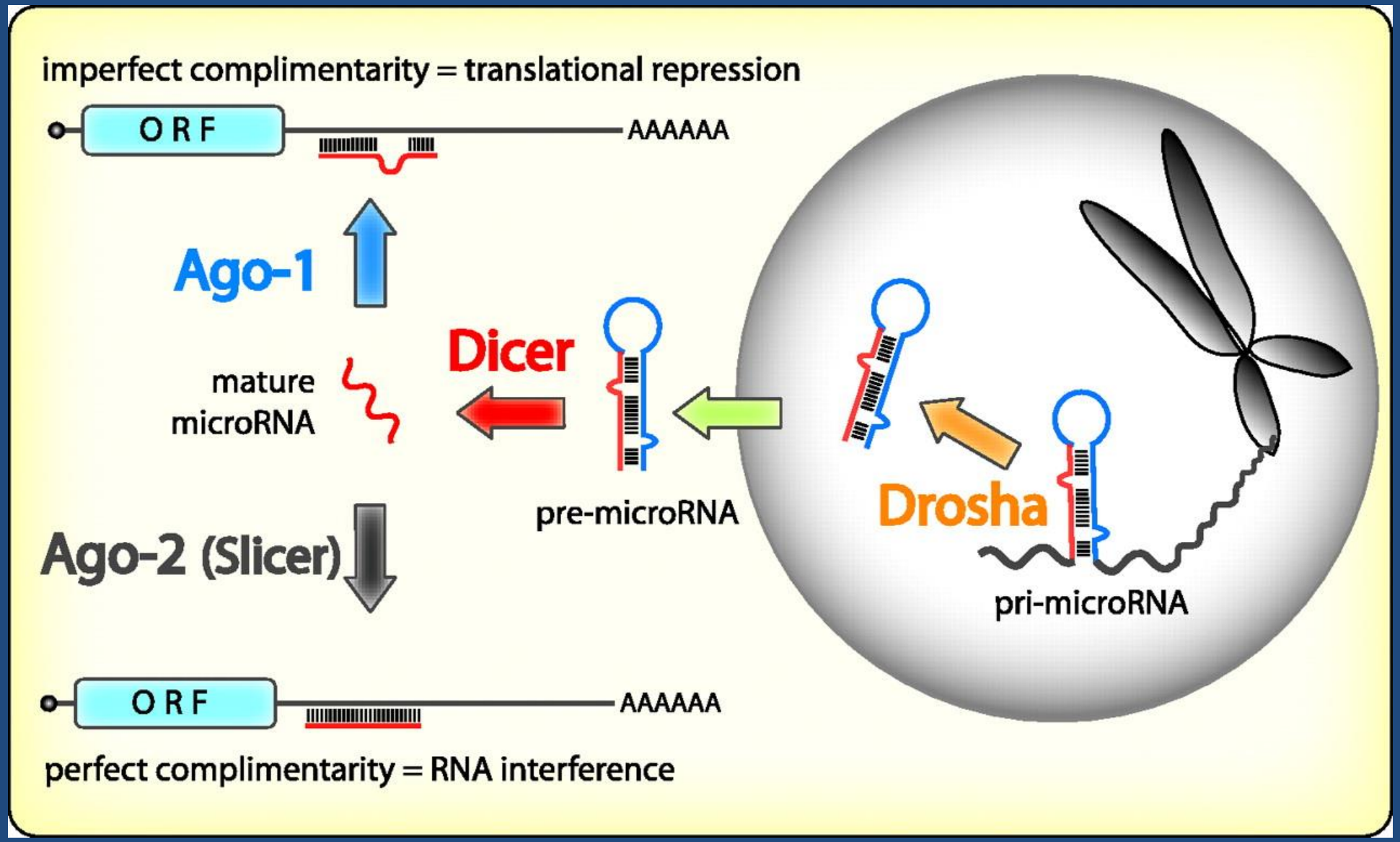
- Dipteran HIF-1α is ~1500 amino acids vs. mammalian ~830, *C. elegans* 719, vs. honeybee and shrimp ~1000
- 3 unique substitutions in *Eurosta* bHLH domain compared with *Drosophila* & honeybee

CONTROL REGION OF A TYPICAL EUKARYOTIC GENE



EPIGENETICS:

- microRNA
- Methylate DNA
- RNA Polymerase [P/deP]
- Histones modified [Me.Ace]
- HDAC / HAT changes

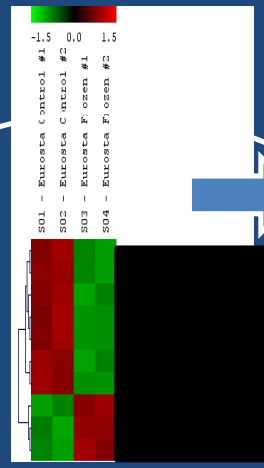


Cuellar TL, McManus MT. J Endocrinol. 187(3):327-332, 2005.

METHODS OUTLINE

- Cold/Freezing
- microRNA Signatures
- *Eurosta, Epiblema*
- Differentially regulated microRNAs in Winter Adaptation
- Future perspectives

Methods

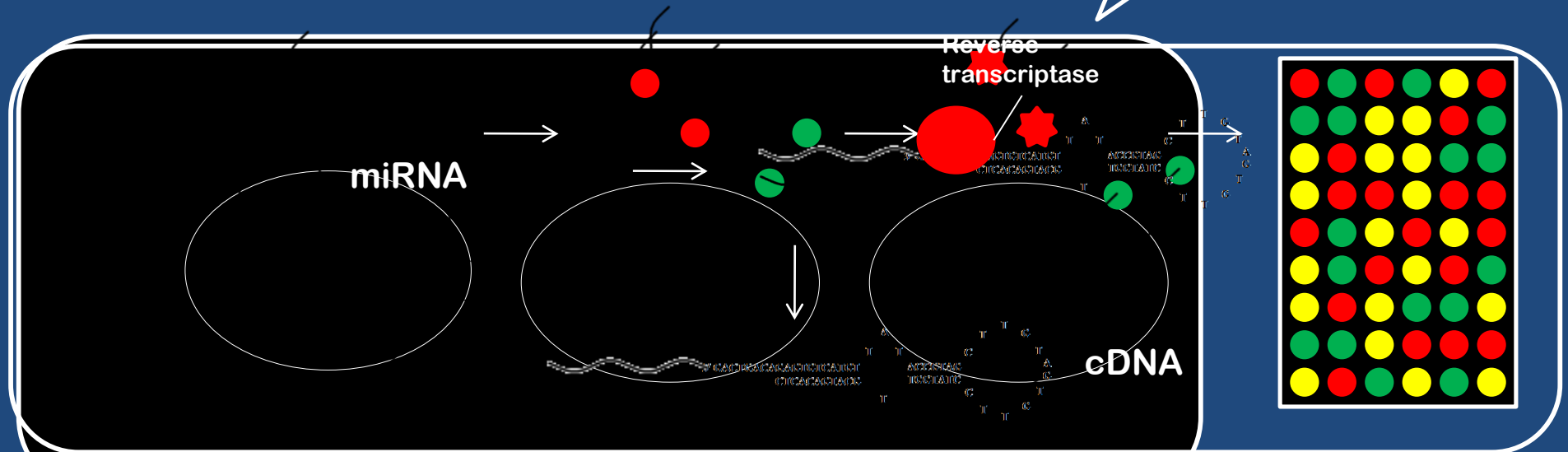


miRNA candidates identified by microarray and literature searches

Homogenization (Polytron)

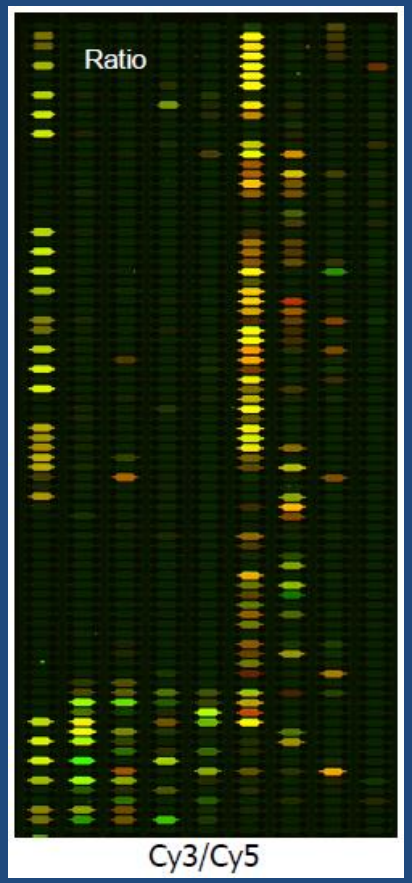
Total RNA isolation

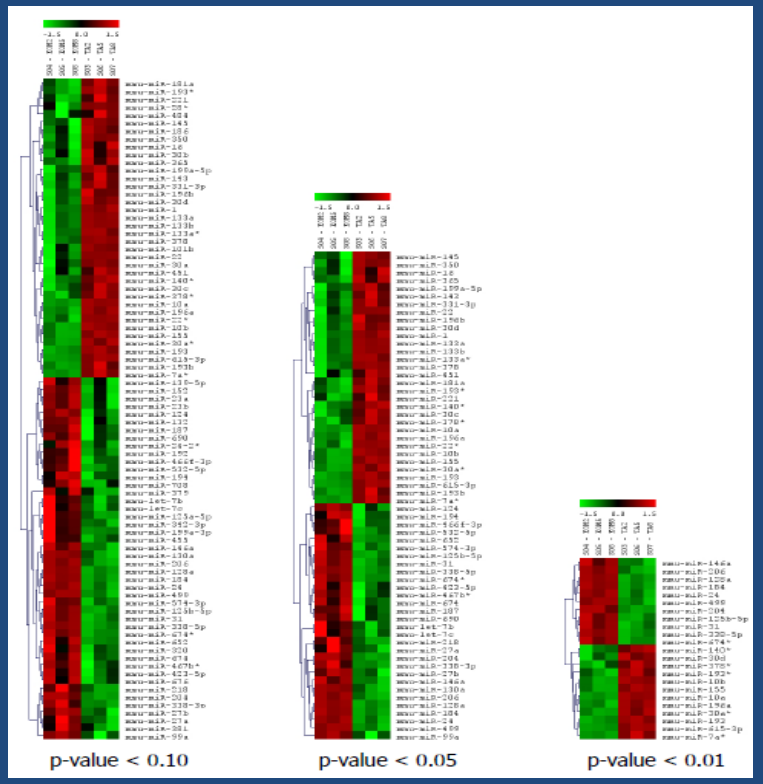
Reverse transcription (Biggar et al., 2011)



Results - microarray

Microarray profiling of *E. solidaginis* at +5 °C and -15 °C to identify miRNA candidates





Comprehensive Physiology. 2013; 3(3): 1283-1308

Molecular Biology of Freezing Tolerance

Kenneth B. Storey[*1] *and Janet M. Storey*[1]

ABSTRACT

Winter survival for many kinds of animals involves freeze tolerance, the ability to endure the conversion of about 65% of total body water into extracellular ice and the consequences that freezing imposes including interruption of vital processes (e.g., heartbeat and breathing), cell shrinkage, elevated osmolality, anoxia/ischemia, and potential physical damage from ice. Freeze-tolerant animals include various terrestrially hibernating amphibians and reptiles, many species of insects, and numerous other invertebrates inhabiting both terrestrial and intertidal environments. Well-known strategies of freezing survival include accumulation of low molecul
cryoprotectants (e.g., glycerol), use of ice nucleating agents/proteins for
of ice growth and of antifreeze proteins that inhibit ice recrystallization, c
anoxia and dehydration. The present article focuses on more recent advar
of the genes and proteins that support freeze tolerance and the metabolic r
involved. Important roles have been identified for aquaporins and transme
move cryoprotectants, heat shock proteins and other chaperones, antic
metabolic rate depression. Genome and proteome screening has revealed
targets that respond to freezing, in particular implicating cytoskeleton rem
facet of low temperature and/or cell volume adaptation. Key regulatory m
versible phosphorylation control of metabolic enzymes and microRNA cor
expression. These help to remodel metabolism to preserve core functions
ergy expensive metabolic activities such as the cell cycle. All of these adv
much more complete picture of life in the frozen state. © 2013 American

Canadian Journal of Zoology. 2012; 90: 456-475

REVIEW / SYNTHÈSE

Insect cold hardiness: metabolic, gene, and protein adaptation[1]

Kenneth B. Storey and Janet M. Storey

Abstract: Winter survival for thousands of species of insects relies on adaptive strategies for cold hardiness. Two basic mechanisms are widely used (freeze avoidance by deep supercooling and freeze tolerance where insects endure ice formation in extracellular fluid spaces), whereas additional strategies (cryoprotective dehydration, vitrification) are also used by some polar species in extreme environments. This review assesses recent research on the biochemical adaptations that support insect cold hardiness. We examine new information about the regulation of cryoprotectant biosynthesis, mechanisms of metabolic rate depression, role of aquaporins in water and glycerol movement, and cell preservation strategies (chaperones, antioxidant defenses and metal binding proteins, mitochondrial suppression) for survival over the winter. We also review the new information coming from the use of genomic and proteomic screening methods that are greatly widening the scope for discovery of genes and proteins that support winter survival.

Cryobiology 65 (2012) 210–214

Contents lists available at SciVerse ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Differential expression of microRNA species in a freeze tolerant insect, *Eurosta solidaginis* ☆

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Freeze tolerance
Eurosta solidaginis
Reversible control of translation
Gene silencing

ABSTRACT

Freeze tolerance in insects is associated with a variety of adaptatio
tants, specialized proteins that regulate ice formation, and energy
press the rates of metabolic processes in the oxygen-limited
microRNAs (miRNAs), small non-coding transcripts that bind to mi
ulation of energy-expensive mRNA translation in frozen insects an
peratures. Expression levels of miRNA species were evaluated
goldenrod gall fly larvae, *Eurosta solidaginis*, using a miRNA m
miR-71, miR-3742, miR-277-3p, miR-2543b and miR-34 were
whereas miR-284, miR-3791-5p and miR-92c-3p rose significan
for two miRNAs, miR-277-3p and miR-284, revealed potential reg
lation and the Krebs cycle. These data constitute the first report
occurs in a freeze tolerant insect and suggest a mechanism for
longed periods of freezing over the winter months, a mechanism
renewed translation of mRNA when temperatures rise and insect

Cryo Letters. 2013 Jan-Feb;34(1):83-9.

Identification of differentially regulated micrornas in cold-hardy insects.

Lyons PJ[1], Poitras JJ, Courteau LA, Storey KB, Morin P Jr.

Author information

Abstract

Freeze tolerance in insects is associated with cryoprotectant synthesis and strong metabolic suppression. Freeze avoidance, an alternative strategy in cold-hardy insects, is also characterized by hypometabolism, but possesses significant cellular and physiological differences when compared with freeze tolerance. We hypothesized that microRNAs, non-coding transcripts that bind to mRNA, could play a role in the regulation of energy-expensive mRNA translation in insects exposed to low temperatures. Expression levels of microRNA species were evaluated during cold acclimation of freeze tolerant Eurosta solidaginis and freeze-avoiding Epiblema scudderiana, comparing control (5 degree C) conditions with larvae given sequential exposures to -5 degree C and -15 degree C. MiR-1 levels were significantly elevated in frozen E. solidaginis larvae at -15 degree C, whereas miR-34 levels were unchanged. MiR-1 and miR-34 levels remained stable in E. scudderiana. These data demonstrate differential microRNA expression in frozen versus control insect larvae and highlight contrasting microRNA signatures between freeze tolerant and freeze avoiding species.

microRNA Deep Sequencing

Epiblema scudderiana – freeze avoiding

- 44 differentially expressed miRNAs between control and cold-exposed larvae
- 21 upregulated miRNAs and 23 downregulated miRNAs

- Potential relevance to cold adaptation: elevated miR-1-3p, miR-92b-3p and miR-133-3p levels and reduced miR-13a-3p and miR-13b-3p

Eurosta solidaginis – freeze tolerant

- Upregulated miRNAs included miR-8-5p, miR-10-5p, miR-31a-5p, miR-34-5p, miR-281-3p, miR-316-5p, miR-317-3p, miR-988 (upregulated)
- Downregulated miRNAs include miR-281-3p, miR-307a-5p, miR-965-3p

- A signature of cold-modulated miRNAs identified suggesting a subset of miRNAs for cold adaptation → CryomiRs

Cold-modulated miRNAs in natural models of hypometabolism

Table 1
MiRNAs modulated in response to cold temperatures. Differentially expressed miRNAs in selected models of freeze tolerance and hibernation. Note: *Eurosta solidaginis*: goldenrod gall fly; *Rana sylvatica*: wood frog (now: *Lithobates sylvaticus*); *Littorina littorea*: common periwinkle; *Myotis lucifugus*: little brown bat; *Ictidomys tridecemlineatus*: thirteen-lined ground squirrel; *Spermophilus parryii*: Arctic ground squirrel.

miRNAs	Conditions	Species	Sample type	Sample size	Method	References
miR-11↓, miR-34↓, miR-71↓, miR-92c-3p↑, miR-276↓, miR-277-3p↓, miR-284↑, miR-2543b↓, miR-3742↓, miR-3791-5p↑	Freezing	*E. solidaginis*	Larvae	2	Microarray	Courteau et al., 2012
miR-1 ↑	Freezing	*E. solidaginis*	Larvae	4	RT-PCR	Lyons et al., 2013
miR-16↓, miR-21↑	Freezing	*R. sylvatica*	Muscle	4	RT-PCR	Biggar et al., 2009
miR-16↑, miR-21↑	Freezing	*R. sylvatica*	Liver	4	RT-PCR	Biggar et al., 2009
miR-1a-1↑, miR-2a↑, miR-29b↑, miR-34a↑, miR-125b↑, miR-133a↑	Freezing	*L. littorea*	Foot muscle	3–4	RT-PCR	Biggar et al., 2012
miR-1a-1↑, miR-29b↑, miR-34a↑	Freezing	*L. littorea*	Hepatopancreas	3–4	RT-PCR	Biggar et al., 2012
miR-1↑, miR-15a↑, miR-20a↑, miR-21↓, miR-23a↑, miR-29b↑, miR-128↑, miR-181b↑, miR-206↑	Hibernation	*M. lucifugus*	Skeletal muscle	3–4	RT-PCR	Kornfeld et al., 2012
miR-24↓	Hibernation	*I. tridecemlineatus*	Heart	4	RT-PCR	Morin et al., 2008
miR-1 ↑, miR-21↑	Hibernation	*I. tridecemlineatus*	Kidney	4	RT-PCR	Morin et al., 2008
miR-24↓, miR-122a↓	Hibernation	*I. tridecemlineatus*	Skeletal muscle	4	RT-PCR	Morin et al., 2008
miR-1↑, miR-142-5p↑, miR-144↑, miR-152↓, miR-184↓, miR-320↓, miR-378 ↓, miR-451↑, miR-486↑	Hibernation	*S. parryii*	Liver	4–6	Microarray/ next-generation sequencing/qRT-PCR	Liu et al., 2010

INSECT COLD HARDINESS

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Methods

Exposure of *E. solidaginis* and *E. scudderiana* larvae to cold stress

Larvae were first incubated at +5 °C (2 weeks) → Sampled
Larvae were then incubated at -5 °C (2 weeks) → Sampled
Larvae were finally incubated at -15 °C (2 weeks→ Sampled

SmallRNA isolation from cold-stressed *E. solidaginis* and *E. scudderiana*

miRVana™ miRNA Isolation Kit (small RNA enriched protocol): 2 extractions of 2 larvae each for +5°C and -15°C

cDNA library preparation and Ion Torrent sequencing

cDNA library prep and barcode tagging of RNA samples
Sequence acquisition
Data trimming (removing barcodes, low Q scores, over/undersized sequences)

MiRNA identification

Alignment of sequencing data with mature *D. melanogaster* miRNA sequences from miRBase

Normalisation and Statistical analysis

Intra-samples variations of read counts were normalized by the Trimmed Mean of M-values (TMM) method
Differential expression was determined by Student's t-test

MiRNA expression profiling via Next-Generation Sequencing: Epiblema

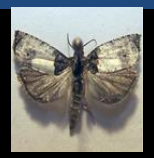


Table 1: Mapping statistics of Ion Torrent reads in cold-stressed vs control *E. scudderiana* larvae.

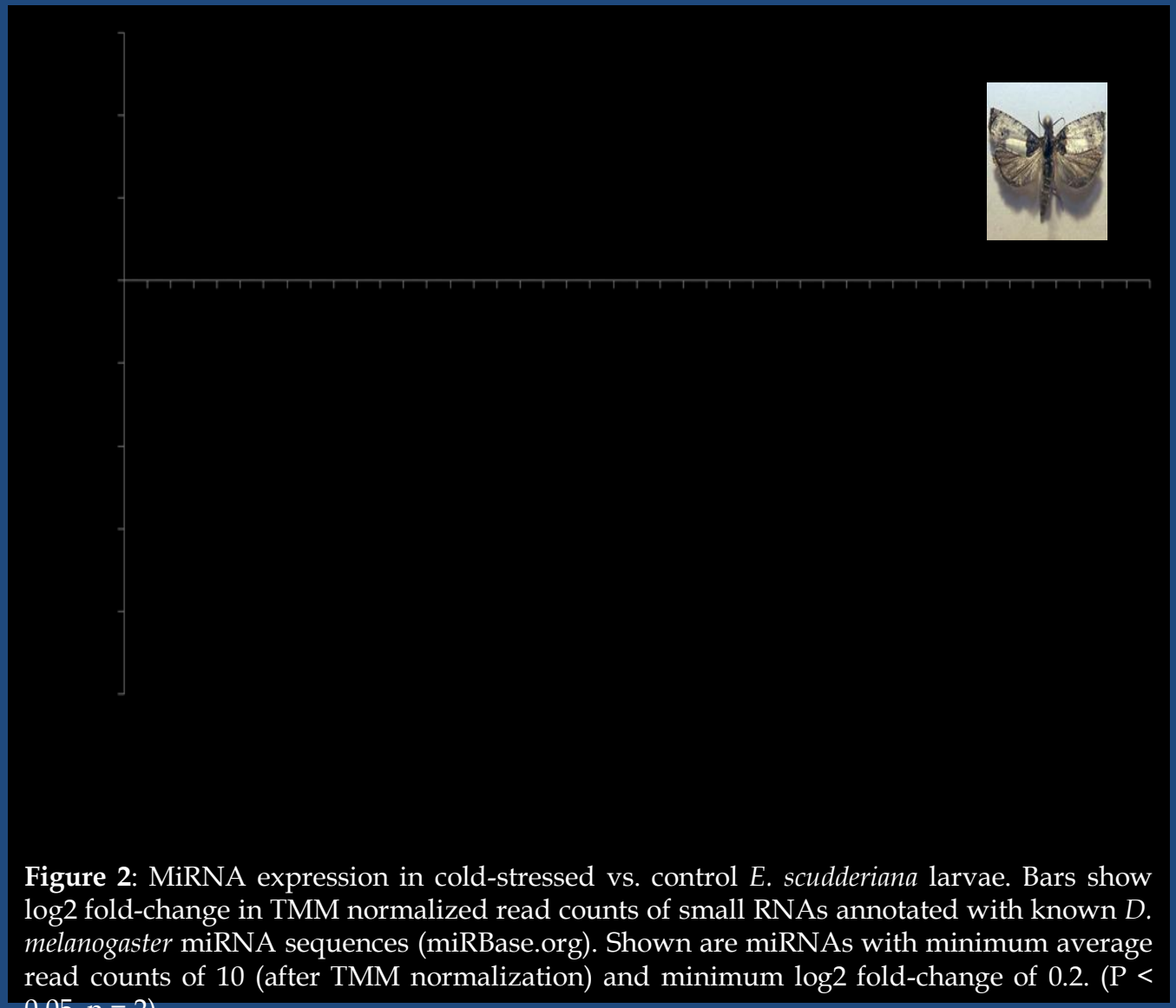


Figure 2: MiRNA expression in cold-stressed vs. control *E. scudderiana* larvae. Bars show log2 fold-change in TMM normalized read counts of small RNAs annotated with known *D. melanogaster* miRNA sequences (miRBase.org). Shown are miRNAs with minimum average read counts of 10 (after TMM normalization) and minimum log2 fold-change of 0.2. ($P < 0.05$, $n = 2$).

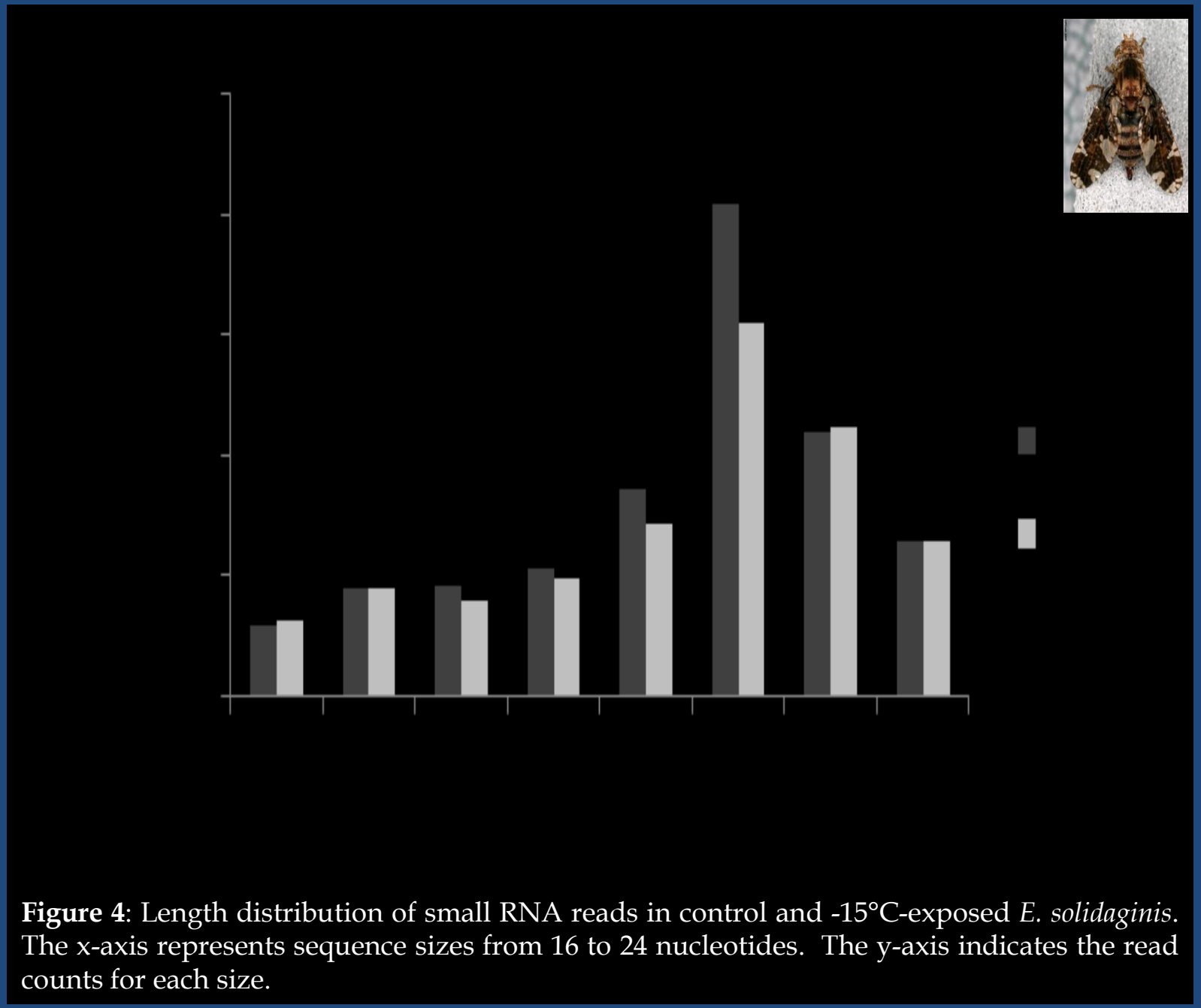


Figure 4: Length distribution of small RNA reads in control and -15°C-exposed *E. solidaginis*. The x-axis represents sequence sizes from 16 to 24 nucleotides. The y-axis indicates the read counts for each size.

Table 2: MiRNAs that are modulated differently in the freeze-avoiding *E. scudderiana* and the freeze-tolerant *E. solidaginis* via next-generation sequencing.

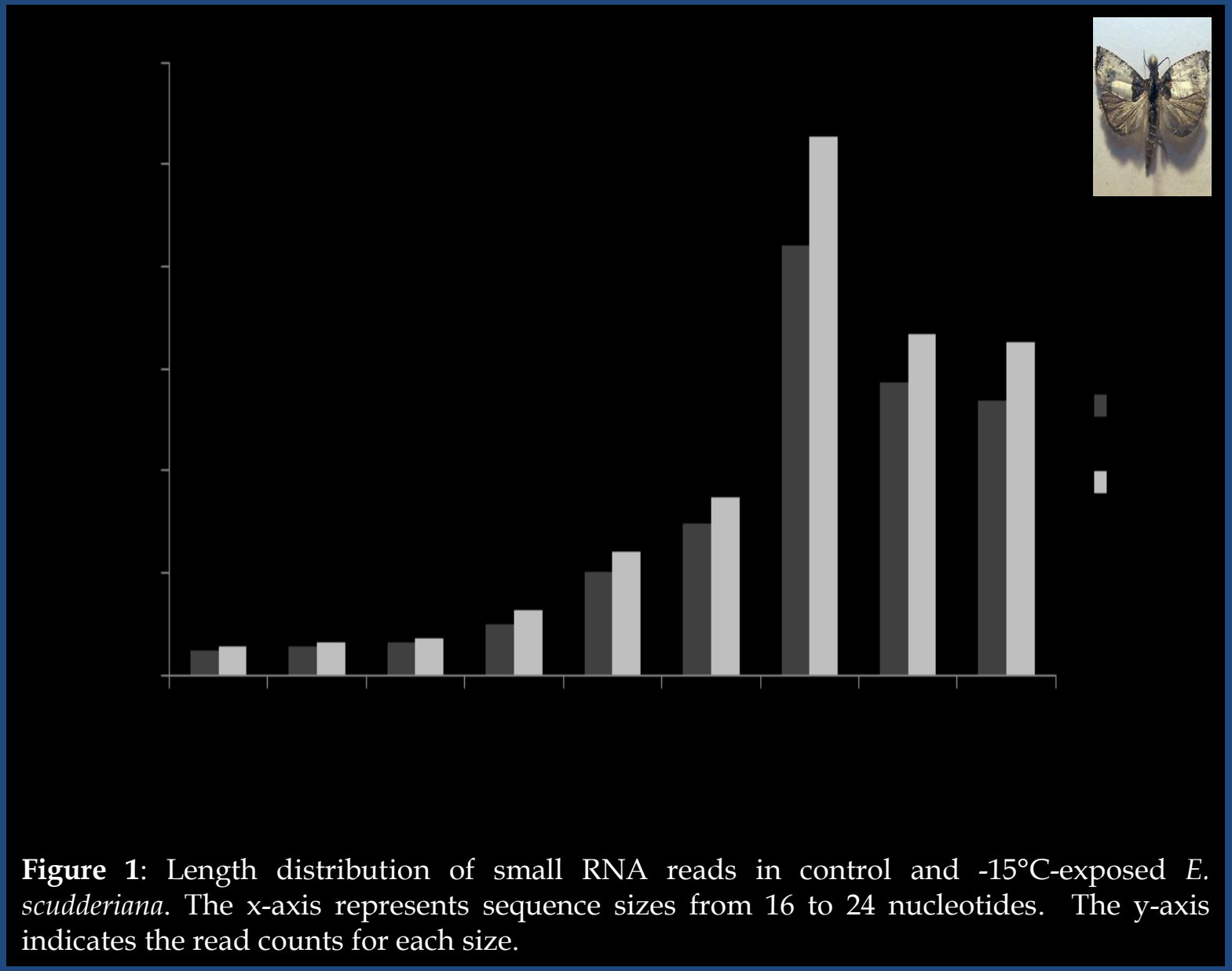


Figure 1: Length distribution of small RNA reads in control and -15°C-exposed *E. scudderiana*. The x-axis represents sequence sizes from 16 to 24 nucleotides. The y-axis indicates the read counts for each size.

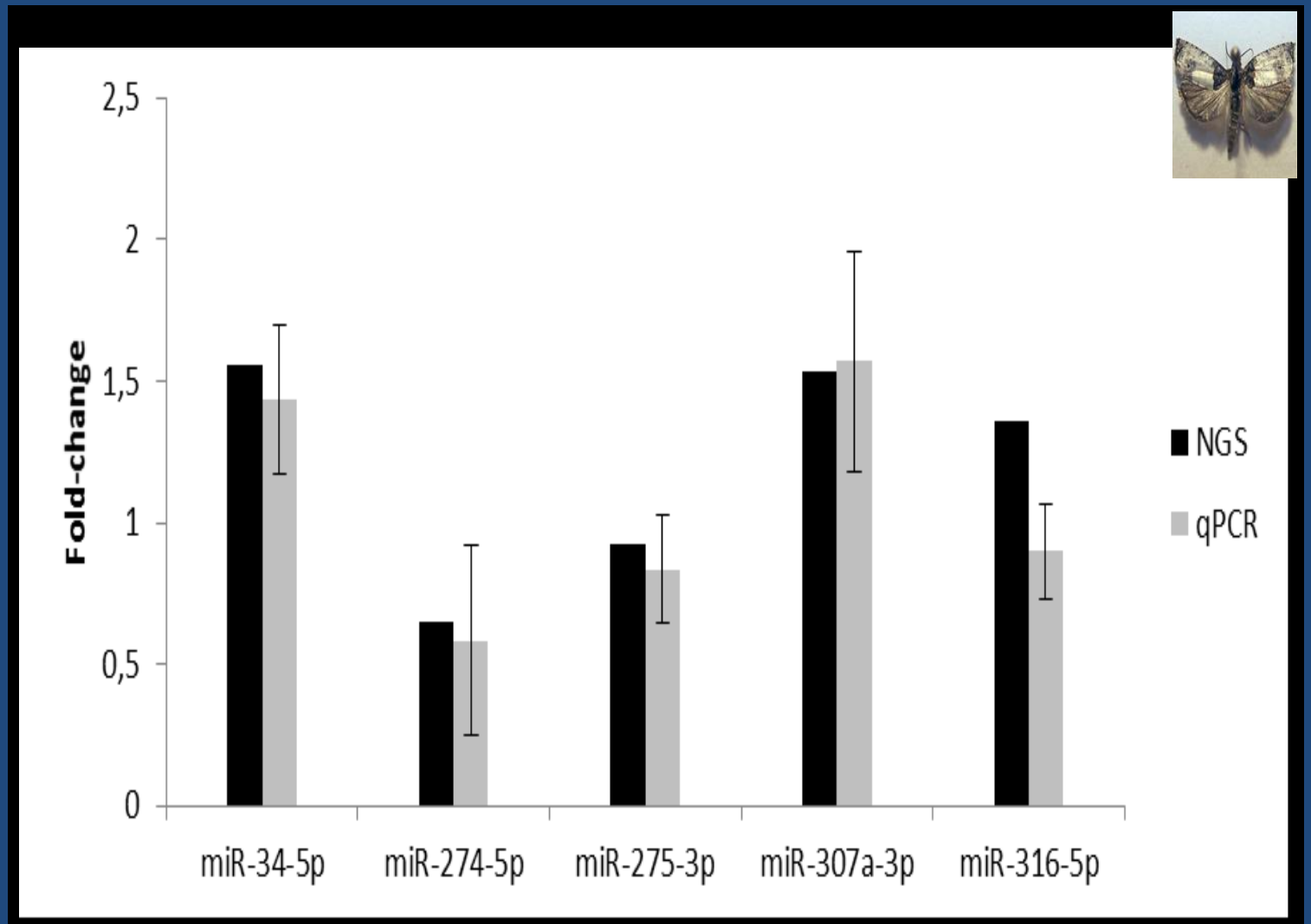


Figure 3: Real-time PCR validation of expression patterns observed for five miRNAs by next-generation sequencing. Relative expression of miR-34-5p, miR-274-5p, miR-275-3p, miR-307a-3p and miR-316-5p quantified by qRT-PCR in control and -15°C-exposed *E. scudderiana*. Data are normalized transcript levels (mean ± SEM, n=4).

CryomiRs:

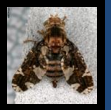


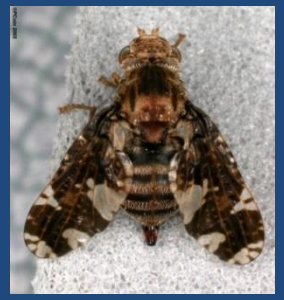
Table 3: A common signature of upregulated and downregulated miRNAs observed in cold-treated *E. solidaginis* and *E. scudderiana* via next-generation sequencing.

Discussion and Conclusion

This project was undertaken to further characterize a miRNA signature solicited at low temperatures in two cold-hardy gall-forming insects. Small enriched RNAs from cold-stressed and control larvae of the freeze-tolerant goldenrod gall fly *Eurosta solidaginis* as well as the freeze-avoiding goldenrod gall moth *Epiblema scudderiana* was characterized via Ion Torrent high throughput sequencing. In *E. scudderiana*, a total of 44 differentially expressed miRNAs were identified between control and cold-exposed larvae with 21 upregulated miRNAs and 23 downregulated miRNAs. Among the most significant changes observed in miRNAs with potential relevance to cold adaptation were elevated miR-1-3p, miR-92b-3p and miR-133-3p levels as well as reduced miR-13a-3p and miR-13b-3p levels in *E. scudderiana* larvae exposed to cold temperatures. Expression values obtained from next-generation sequencing were also validated by a quantitative PCR approach for five miRNAs; miR-34-5p, miR-274-5p, miR-275-3p, miR-307a-3p and miR-316-5p. Interestingly, a next-generation sequencing-based approach was also undertaken in the freeze-tolerant *E. solidaginis* and revealed miRNAs that displayed similar expression profiles as the ones observed in cold-exposed *E. scudderiana*. These include miR-8-5p, miR-10-5p, miR-31a-5p, miR-34-5p, miR-281-3p, miR-316-5p, miR-317-3p, miR-988 (upregulated) and miR-281-3p, miR-307a-5p, miR-965-3p (downregulated). This common signature of cold-modulated miRNAs suggest that a sub-set of miRNAs are differentially expressed in insects during cold adaptation. Investigating this signature in other animal models of cold adaptation, including mammalian hibernators that interestingly also exhibit miR-1 upregulation, will further put the light on cold-responsive miRNAs or CryomiRs.

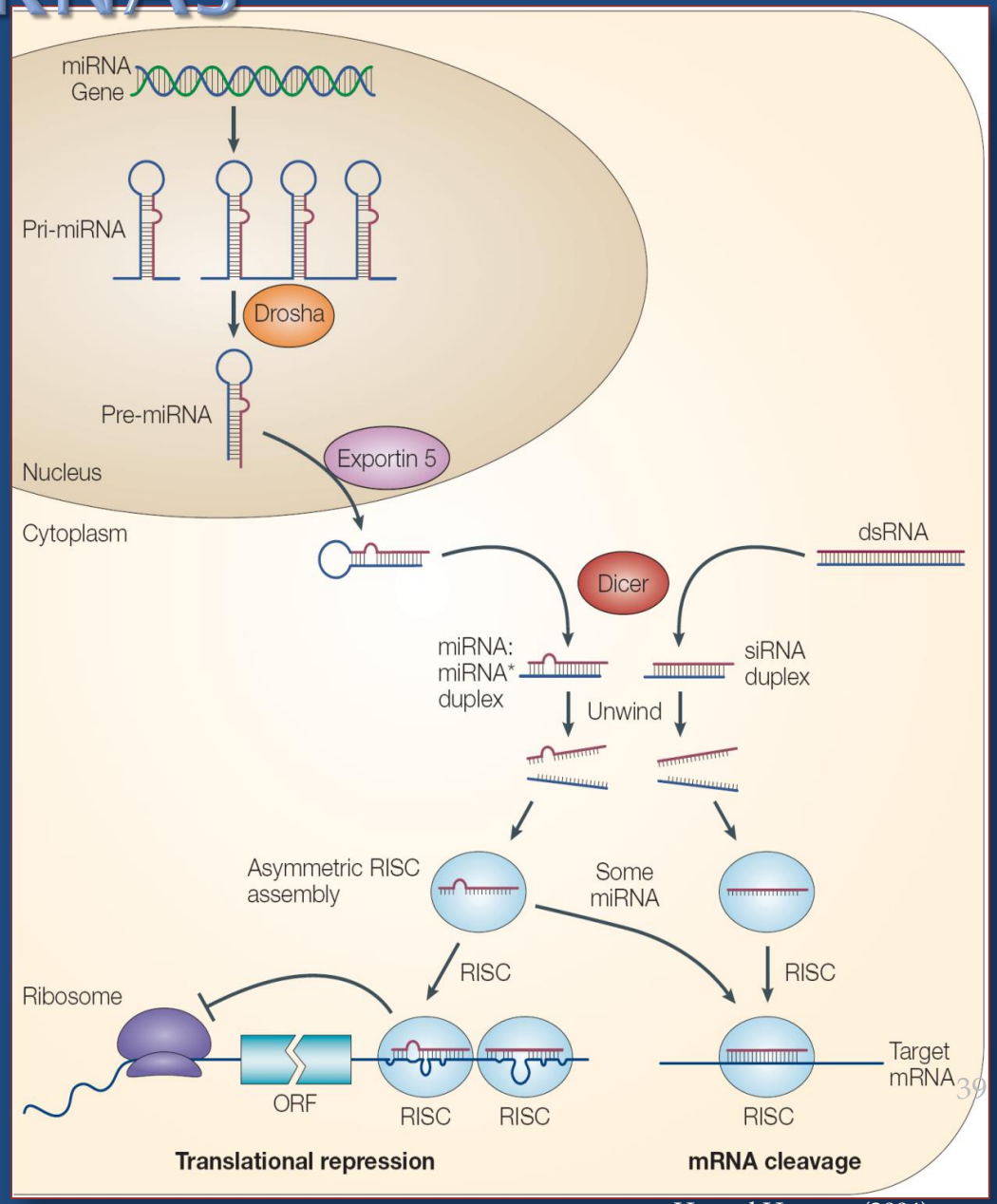
Overview of insect cold hardiness

Characteristics	*E. solidaginis*	*E. scudderiana*
Freeze tolerance strategy	Yes	No
Freeze avoidance strategy	No	Yes
Intracellular ice formation	No	No
Extracellular ice formation	Yes	No
Metabolic depression	Yes	Yes



miRNAs

- Short, non-coding, RNAs
- 18-23 nucleotides in length
- Involved in translational repression
- Differentially expressed at low temperatures in selected amphibians and hibernators



He and Hannon (2004)

microRNA Summary

1. MiRNA signatures were solicited at low temperatures in two cold-hardy gall-forming insects
2. In *E. scudderiana*, a total of 44 differentially expressed miRNAs were identified between control and cold-exposed larvae with 21 upregulated miRNAs and 23 downregulated miRNAs.
3. Potential relevance to cold adaptation: elevated miR-1-3p, miR-92b-3p and miR-133-3p levels as well as reduced miR-13a-3p and miR-13b-3p levels in *E. scudderiana* larvae exposed to cold temperatures.
4. in the freeze-tolerant *E. solidaginis* similar miRNAs were determined These include miR-8-5p, miR-10-5p, miR-31a-5p, miR-34-5p, miR-281-3p, miR-316-5p, miR-317-3p, miR-988 (upregulated) and miR-281-3p, miR-307a-5p, miR-965-3p (downregulated).
5. This common signature of cold-modulated miRNAs suggest that a sub-set of miRNAs are differentially expressed in insects during cold adaptation.
6. Investigating this signature in other animal models of cold adaptation on cold-responsive miRNAs or CryomiRs.

Cold-modulated miRNAs in natural models of hypometabolism

Table 1
MiRNAs modulated in response to cold temperatures. Differentially expressed miRNAs in selected models of freeze tolerance and hibernation. Note: *Eurosta solidaginis*: goldenrod gall fly; *Rana sylvatica*: wood frog (now: *Lithobates sylvaticus*); *Littorina littorea*: common periwinkle; *Myotis lucifugus*: little brown bat; *Ictidomys tridecemlineatus*: thirteen-lined ground squirrel; *Spermophilus parryii*: Arctic ground squirrel.

miRNAs	Conditions	Species	Sample type	Sample size	Method	References
miR-11↓, miR-34↓, miR-71↓, miR-92c-3p↑, miR-276↓, miR-277-3p↓, miR-284↑, miR-2543b↓, miR-3742↓, miR-3791-5p↑	Freezing	*E. solidaginis*	Larvae	2	Microarray	Courteau et al., 2012
miR-1 ↑	Freezing	*E. solidaginis*	Larvae	4	RT-PCR	Lyons et al., 2013
miR-16↓, miR-21↑	Freezing	*R. sylvatica*	Muscle	4	RT-PCR	Biggar et al., 2009
miR-16↑, miR-21↑	Freezing	*R. sylvatica*	Liver	4	RT-PCR	Biggar et al., 2009
miR-1a-1↑, miR-2a↑, miR-29b↑, miR-34a↑, miR-125b↑, miR-133a↑	Freezing	*L. littorea*	Foot muscle	3–4	RT-PCR	Biggar et al., 2012
miR-1a-1↑, miR-29b↑, miR-34a↑	Freezing	*L. littorea*	Hepatopancreas	3–4	RT-PCR	Biggar et al., 2012
miR-1↑, miR-15a↑, miR-20a↑, miR-21↓, miR-23a↑, miR-29b↑, miR-128↑, miR-181b↑, miR-206↑	Hibernation	*M. lucifugus*	Skeletal muscle	3–4	RT-PCR	Kornfeld et al., 2012
miR-24↓	Hibernation	*I. tridecemlineatus*	Heart	4	RT-PCR	Morin et al., 2008
miR-1 ↑, miR-21↑	Hibernation	*I. tridecemlineatus*	Kidney	4	RT-PCR	Morin et al., 2008
miR-24↓, miR-122a↓	Hibernation	*I. tridecemlineatus*	Skeletal muscle	4	RT-PCR	Morin et al., 2008
miR-1↑, miR-142-5p↑, miR-144↑, miR-152↓, miR-184↓, miR-320↓, miR-378 ↓, miR-451↑, miR-486↑	Hibernation	*S. parryii*	Liver	4–6	Microarray/ next-generation sequencing/qRT-PCR	Liu et al., 2010

Conclusion and future perspectives

- miR-210, miR-1, miR-284 and miR-34 were amplified and measured for the first time in a freeze tolerant insect

- Expression of this miR signature in a freeze-avoiding insect

- Characterization of miR targets in our insect models